we have proposed the dynamical event and plausible mechanism of complex formation of Gal3p and Gal1p with Gal80p at the molecular level.

2590-Pos Board B20

Death Effector Domain Flexibility in Mediating Protein-Protein Interactions

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PED/PEA-15 (phosphoprotein enriched in diabetes/astrocytes, 15 kD) is a small, non-catalytic, death-effector domain (DED) containing protein, that is widely expressed in different tissues and highly conserved among mammals. PED/PEA-15 has been found to interact with several protein targets in various pathways, including FADD and procaspase-8 (apoptosis), ERK1/2 (cell cycle entry), and PLD1/2 (diabetes). We have previously reported a surprising conformational change of PED/PEA-15 DED upon interaction with ERK2 using NMR dynamics and residual dipolar coupling (RDC) data. In the complex, PED/PEA-15 utilizes helices α1, α5, and α6 of the DED, in addition to the C-terminal tail, to binding to ERK2, while helices α2, α3, and α4 are highly flexible in the complex, and adopt a distinct relative orientation comparing to the free-form conformation. We have additionally modeled the PED/PEA-15 conformations in the ERK2 complex using CS/RDC-Rosetta protocol. Based on our NMR model of PED/PEA-15 complex with ERK2, we propose that the conserved charge triad motif on DED surface, comprising of D19-R72-D74L, which is located at a hinge position between these two dynamically distinct segments, mediates the necessary conformational changes to accommodate ERK2 binding. We believe that various conformations of the DED, facilitated by large number of surface polar interactions, may attribute to its ability to interact with structurally and functionally diverse proteins.

2591-Pos Board B21

Inhibition of GPR18 through Docking of known Antagonists using a Homology Model

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The Class A G-protein coupled receptor, GPR18, is a newly de-orphanized cannabinoid receptor that binds the endogenous ligand, N-arachidonoyl glycine (NAGly). As part of a larger project to model the interaction of ligands with GPR18, we undertook the conformational analysis of NAGly. Because NAGLY is an arachidonic acid derivative and arachidonic acid has been shown to be highly flexible, we began by investigating the conformational flexibility of this endogenous ligand. To this end, the Conformational Memories (CM) method was employed. This method combines Monte Carlo exploration of the dihedral angle and bond angle space with simulated annealing (MC/SA) to determine the range of values that each dihedral angle and bond angle is capable of exploring in a broad temperature range (Whitnell J Comp Chem 2007). Similar to previously reported CM results for arachidonic acid (Barnett-Norris J Med Chem 1998), NAGly's conformers could be divided into four groups: linear, U-shape, J-shape, and helical. These results will be used to explore the binding site interactions of NAGly at GPR18.

2592-Pos Board B22

Targeting Apicomplexan Atg8 for Rational Drug Design

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Apicomplexan pathogens present significant health and economic burdens globally. Species of *Plasmodium*, the causative agent of malaria, result in more than half a million deaths annually, with the primary victims being children under the age of five. Other organisms in this phylum present a threat to immunocompromised individuals (e.g., *Toxoplasma gondii*, *Cryptosporidium* spp.) or to the productivity of the livestock industry (e.g., *Eimeria* spp., *Neospora caninum*). Autophagy is the process by which cells recycle intracellular material by encapsulating them in a double membrane bound vesicle, the autophagosome, which then fuses with the lysosome. The autophagy pathway has been shown to be essential to parasite survival in both *Plasmodium* and *Toxoplasma*. Autophagy-related protein 8 (Atg8), which is required for the formation of the autophagosomal membrane, is a ubiquitin-like protein whose conjugation pathway is conserved in Apicomplexa. Within Apicomplexans, Atg8 has a conserved loop region not present in human homologues. In *Plasmodium falciparum*, this loop was shown to be essential to the interaction between Atg8 and its E2 conjugating enzyme, Atg3, and may represent a viable pan-Apicomplexan drug target. We are pursuing this possibility using x-ray crystallography, SPR, and virtual library screening to identify small compound drug leads which can then be chemically diversified and optimized. Having solved the structure of PfAtg8, we performed two screens against this protein: a screen of the MMV Malaria box of compounds and a virtual library screen. We pursued hits from both screens and tested them via SPR against five pairs of Atg3 and Atg8 homologues: *Plasmodium falciparum*, *Homo sapiens*, *Cryptosporidium parvum*, *Eimeria tenella*, and *Neospora caninum*. We have now also obtained protein crystals for the Atg8 homologues in *E. tenella*, *C. parvum*, and *N. caninum*, which will facilitate future virtual library screens against these specific proteins.

2593-Pos Board B23

Probing Conformational Change in the First Actin-Binding Domain of Dystrophin

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We have used time-resolved EPR and fluorescence to resolve structural transitions of dystrophin upon actin binding. Dystrophin (Dys) is a muscle cytoskeletal protein that binds to filamentous actin (F-actin) and the dystroglycan complex in the sarcolemmal membrane. Dys acts to dissipate mechanical forces generated during the contraction and relaxation of muscle thereby maintaining sarcolemmal membrane integrity and protecting from tears. The protein-protein interactions and allostery underlying this function of Dys have not been well studied in the context of conformational change and thermodynamics, partly because acquisition of structural and thermodynamic detail on large and flexible proteins is difficult. Two techniques capable of measuring large-scale conformational changes are dipolar electron-electron resonance (DEER) and time-resolved fluorescence resonance energy transfer (TR-FRET). Using a combination of DEER and TR-FRET, we placed a single label (nitroxide or fluorescent) in each CH domain Dys ABD1 and subsequently measured the interprobe distance to assess conformational change upon association with F-actin. To probe the allosteric network of Dys ABD1, we also subjected the protein to differential scanning calorimetry.

2594-Pos Board B24

The Interaction between L-PGDS and its Substrates or Products, as Determined by Isothermal Titration Calorimetry and NMR

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Lipocalin-type Prostaglandin D synthase (L-PGDS) catalyzes the isomerization of prostaglandin H_2 (PGH_2) to produce prostaglandin D_2 (PGD_2), which acts as a somnogen in the brain. This enzyme belongs to the lipocalin superfamily which consists of transporter proteins for lipophilic substances in the extracellular space. Our previous studies suggested that L-PGDS is comprised of a β-barrel structure with a hydrophobic pocket and the active thiol group of Cys65 is located in this pocket and faces the inside of the pocket. A number of studies of L-PGDS, as a drug target for treating sleep disorders, have been reported, in attempts to understand its catalytic mechanism, and several substrate recognition models of L-PGDS have been proposed. However, details of the mechanism by which L-PDGS recognizes its substrates and products are obscure, since essential information, such as its binding affinity and stoichiometry of the interactions between L-PGDS and its substrate and product remains unclear. To address this, the binding properties of the molecule were examined by isothermal titration calorimetry (ITC) and NMR experiments. The results of the ITC measurements revealed that, not only the substrate analog, but also the product bind to L-PGDS in a stoichiometry of 2 to 1 and that L-PGDS possesses two binding sites (high and low affinity sites). In addition, NMR titration and ITC experiments of L-PGDS mutants indicated that the active Cys65 residue is located at the high affinity-binding site and plays a critical role in the binding of the substrate and product to L-PGDS.

2595-Pos Board B25

Investigating Interactions between the Lectin-Like Domain of Thrombomodulin and Complement Component 3

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Protein-protein interactions are vital to the proper functioning of numerous biological systems. Thrombomodulin (TM) is a protein that is involved in the down-regulation of coagulation induced by the clotting protein thrombin. Complement component 3 (C3) is a vital component of the complement system, which is involved in innate immunity against bacteria and viruses. However, dysregulation of C3 can lead to the degradation of host cells. Evidence suggests that the lectin-like domain of TM (TMD1) may interact with active C3 (C3b) to inactivate it, thus preventing host cell degradation. The research conducted